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A Cre/*loxP*-Deleter Transgenic Line in Mouse Strain 129S1/SvImJ

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Summary: A Cre recombinase expression cassette was inserted into the X-linked *Hprt* locus by gene targeting in a mouse embryonic stem (ES) cell line isogenic to strain 129S1/SvImJ (129S1), then the transgene was introduced into 129S1 mice through ES cell chimeras. When females hemizygous for this transgene were mated to males carrying a neomycin selection cassette flanked by *loxP* sites, the cassette was always excised regardless of *Cre* inheritance and without detectable mosaicism. The usefulness of this "Cre-deleter" transgenic line is in its efficiency and defined genetic status in terms of mouse strain and location of the transgene. genesis 32: 199–202, 2002. © 2002 Wiley-Liss, Inc.

Key words: gene targeting; deleter; transgene; Cre recombinase; *Hprt*; transgene

The bacteriophage Cre/*loxP* recombination system (Sternberg and Hamilton, 1981) is now of major importance in modification of the mouse genome through the use of embryonic stem (ES) cells (Stricklett *et al.*, 1999). Cre recombinase excises DNA segments flanked by *loxP* sites (floxed) and in the simplest application is used for the excision of selection cassettes included in gene-targeting constructs. This application is important because the cassettes can unintentionally affect the expression of neighboring genes. Although recombinant ES cell clones can be subjected to transient Cre expression to induce excision of selection cassettes prior to chimera production, it can be more convenient to mate ES cell chimeras to Cre-deleter transgenic lines to obtain offspring with the excision event. However, if the mouse strains of the Cre-deleter transgenic and ES cell lines are not matched, then it is not possible to immediately maintain the genetic modification in an inbred strain. Many ES cell lines being used are substrains of strain 129. Two commonly used lines are CJ7 (Swiatek and Gridley, 1993) and W9.5 (Lau *et al.*, 1994; Szabó and Mann, 1994) of strain 129S1/Sv-$p^+Tyr^+Kitl^{Sl\text{-}J}$/+ (Stevens, 1970) (Jackson Laboratory Stock No. 000090). Both lines are $Kitl^+/Kitl^+$ and are therefore isogenic to strain 129S1/SvImJ (129S1) (Jackson Laboratory Stock No. 002448) (Festing *et al.*, 2001). Here we describe a Cre-deleter targeted transgenic line in strain 129S1 in which a *Cre* expression cassette has been inserted into the X-linked hypoxanthine guanine phosphoribosyl transferase *(Hprt)* gene. This locus was chosen for integration since a very high frequency of targeted clones can be obtained through dual selection, and, as *Hprt* is constitutively expressed, the region is favorable for expression (Bronson *et al.*, 1996, Cvetkovic *et al.*, 2000; Evans *et al.*, 2000; Guillot *et al.*, 2000; Hatada *et al.*, 1999). Mating males carrying a floxed segment to females hemizygous for the *Cre* transgene results in excision without mosaicism regardless of *Cre* inheritance.

A total of five G418 and 6-thioguanine dually resistant clones were obtained and were individually expanded. All five were killed under HAT selection, demonstrating all had lost *Hprt* activity and indicating all had been targeted. This was confirmed by Southern blot—all clones displayed the shift in band size expected on targeting at *Hprt* (Fig. 1B). Germ line transmission was obtained with one clone. The line produced is designated $Hprt^{tm1(CAG.cre)MdCoh}$, hereafter abbreviated as $Hprt^{cre}$. The mutation is now maintained by $Hprt^{cre}/Hprt^{cre}$ ♀ × $Hprt^{cre}/Y$♂ matings in strain 129S1, while $+/Hprt^{cre}$ females for use in excision are produced by 129S1 +/+ ♀ × $Hprt^{cre}/Y$♂ matings.

From +/+, $Hprt^{cre}/+$ ♀ × $R26R^{neo}/R26R^{neo}$, +/Y♂ matings, all of 72 embryos at 11.5 to 13.5 days postcoitum (dpc) displayed intense and ubiquitous activity of LacZ (Fig. 2A). Half of these embryos would be expected not to have inherited $Hprt^{cre}$, therefore excision of the floxed *neo* sequence of $R26R^{neo}$ appeared to have occurred regardless of $Hprt^{cre}$ inheritance. This was confirmed by Southern blot—in none of six $Hprt^{cre}/+$, $R26R^{lacZ}/+$ and six +/+, $R26R^{lacZ}/+$ embryos was the $R26R^{neo}$ allele detected (lanes 10–15, Fig. 2B). In addi-

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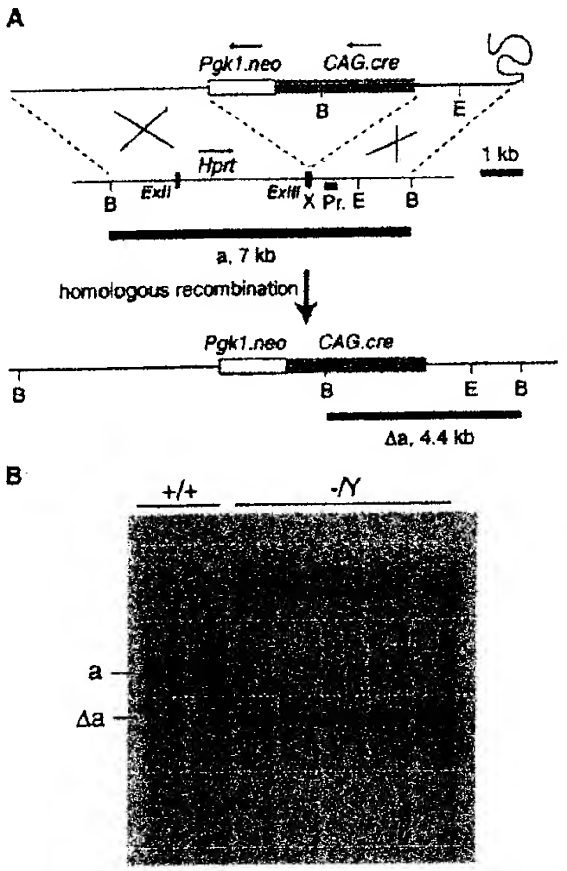

FIG. 1. Insertion of CAG-*Cre* at *Hprt*. **(A)** Targeting vector. B, *Bam* HI; X, *Xho* I; E, *Eco*R I; Pr., probe. Arrows indicate direction of transcription. **(B)** Southern blot. +/+ lanes are DNA samples from two female 129S1 mice. -/Y lanes are the five recombinant clones obtained. An amount of 10 μg of genomic DNA was loaded in each lane. The relative intensity of -/Y relative to +/+ bands indicates that in each of the five recombinant clones a single copy of the transgene is present.

tion, given the detection limit of this blot it appeared that no embryo was mosaic for the $R26R^{neo}$ allele. The same results, involving similar numbers of embryos, were obtained for another targeted mutation in which a floxed *neo* cassette was introduced near the *H19* gene (Szabó *et al.*, 2002). These data indicate that oocytes of $Hprt^{cre}$/+ females have sufficient stored Cre to excise floxed DNA segments at the zygote or early cleavage stages, and that rarely, if ever, is the floxed *neo* cassette not excised. Hence, this new transgenic line is apparently as efficient as the CAG-*Cre* transgenic line produced by zygote injection in strain C57BL/6 (Sakai and Miyazaki, 1997).

From the reciprocal mating, $R26R^{neo}/R26R^{neo}$, +/+ ♀ +/+, $Hprt^{cre}/Y$♂, of 19 female and 22 male pups assayed for LacZ activity, all females were positive, whereas all males were negative. This was the result expected for X chromosome transmission of the $Hprt^{cre}$

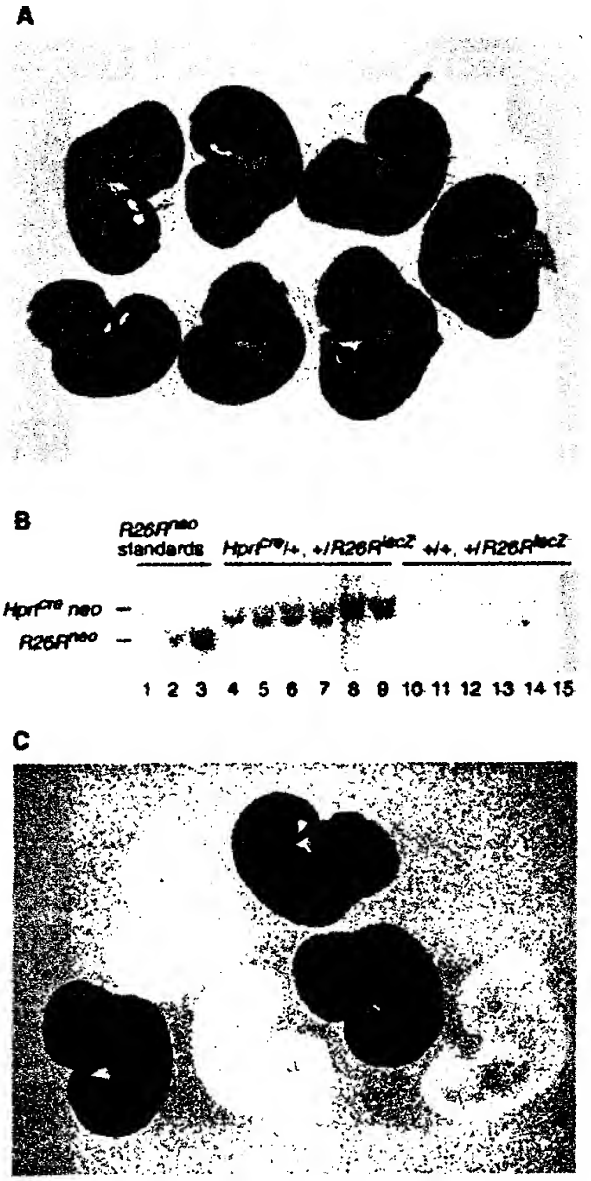

FIG. 2. Efficiency of excision mediated by $Hprt^{cre}$ **(A)** 11.5 dpc siblings of an +/+, $Hprt^{cre}$/+ ♀ × $R26R^{neo}/R26R^{neo}$, +/Y♂ mating stained for LacZ activity—all stained dark blue **(B)** Genomic DNA isolated from the heads of 14.5 dpc embryos obtained from the same type of mating as in (A) was digested with *Eco*R V then probed for *neo* in a Southern blot. All lanes were loaded with 10 μg of DNA. Lane 3 is DNA from a $R26R^{neo}/R26R^{neo}$, +/+ mouse, and lanes 2 and 1 are five times and 25 times dilutions, respectively, of this DNA with +/+, +/+ DNA. Lanes 1–3 are standards showing the 3.8-kb $R26R^{neo}$ band (Soriano, 1999). This band is seen only when the floxed *neo* cassette is not excised. In lanes 4–9, the larger band is from the *neo* gene of the $Hprt^{cre}$ transgene. The 3.8-kb $R26R^{neo}$ band was not detected in lanes 4–15. For these embryos, an 8% level of mosaicism would have resulted in a 3.8-kb band intensity equivalent to that in lane 1 **(C)** 11.5 dpc siblings of an $R26R^{neo}/R26R^{neo}$, +/+ ♀ × +/+, $Hprt^{cre}/Y$♂ mating stained for LacZ activity. The three that stained are presumptive females ($R26R^{neo}$/+, +/$Hprt^{cre}$), whereas the three that did not stain are presumptive males ($R26R^{neo}$/+, +/Y).

allele. At 11.5 dpc, presumptive female embryos displayed intense and ubiquitous X-gal staining as was observed in the reciprocal mating, whereas presumptive males showed no staining (Fig. 2C).

We have described a Cre—deleter transgenic line in strain 129S1 that is useful for its efficiency-excision that is apparently complete at the first stages of development although occasional mosaicism cannot be ruled out, and for its defined genetic status in terms of mouse strain and location of the transgene.

METHODS

Targeting Vector

A 7-kb *Bam* HI fragment containing *Hprt* exons II and III was excised from an 18.6-kb genomic clone derived from CC1.2 ES cells of strain 129 (Deng and Capecchi, 1992) and ligated into the *Bam* HI cloning site of the BAC cloning vector pBeloBAC11 (Wang *et al.*, 1997) to give pBAC-$Hprt^{Bam}$. A Cre expression cassette in which *Cre* is driven by the cytomegalovirus early enhancer/chicken β-globin promoter was excised from the pCAG-*Cre* vector (Sunaga *et al.*, 1997) using *Sal* I and *Hind* III and inserted upstream of the promoter in the phosphoglycerate kinase 1 *(Pgk1)* promoter-*neomycin*-bovine polyA positive selection cassette. These cassettes were then excised as one fragment and inserted into the unique *Hprt* exon III *Xho* I site of pBAC-$Hprt^{Bam}$ to give pBAC-$Hprt^{Bam}$.*neo.cre* (Fig. 1A). Insertion at this site results in *Hprt* inactivation (Deng and Capecchi, 1992). This BAC subcloning system was used since we had difficulty in obtaining ligation into the *Hprt Xho* I site using plasmids. However, we since found that this step was readily achieved when the bacterial strain STBL2 (Stratagene, La Jolla, CA) was used for transformation. The probe used to identify homologous recombinants was a 0.25-kb *Rsa* I fragment excised from the *Xho* I-*Eco*R I-fragment (Fig. 1A). The CAG-*Cre* cassette was chosen since this has been shown in a transgenic line, produced by zygote injection in strain C57BL/6, to perform the function we were seeking—excision of a floxed segment during early cleavage regardless of *Cre* inheritance (Sakai and Miyazaki, 1997).

Gene Targeting in ES Cells

The pBAC-$Hprt^{Bam}$.*neo.cre* targeting vector was linearized at the end of the long arm using *Srf* I and introduced into W9.5 ES cells by electroporation using standard conditions. Four electroporations were performed, using 25 μg of DNA and 10^7 cells in each, and the total number of cells plated onto four 10 cM plates with STO fibroblast feeder cells. ES cells were selected with 175 μg/mL active weight G418 for five days, then G418 at this same concentration plus 10 μg/mL 6-thioguanine for a further seven days, after which dually resistant clones were picked and expanded.

Germ-Line Transmission of *Cre*

Targeted clones were injected into (MB6—see below x C57BL/6J-Tyr^{c-2J})F_2 blastocysts to produce chimeras. Chimeras were mated to 129S1/SvImJ females to obtain germ-line transmission and maintain *Cre* in this strain. Primers within the *Cre* sequence were used to identify mice positive for $Hprt^{cre}$—5′ to 3′, upper, TGCTGTTTCACTGGTTGTGCGGCG, and lower, TGCCTTCTCTACACCTGCGGTGCT. Homozygous females were identified by the lack of PCR amplification of a 0.22-kb product spanning the *Hprt Xho* I insertion site—primers, 5′ to 3′, upper, CCTGATTTTATTTCTATAGGACTGAAAGAC, lower, TAAGTAATTATACTTACACAGTAGCTCTTC.

Assessment of Excision Efficiency

The efficiency of Cre-mediated excision of floxed DNA in +/$Hprt^{cre}$ female mice was assessed by mating them to males homozygous for the *Rosa26* Cre-Reporter ($R26R^{neo}$) transgene. In this line, *lacZ* expression can be induced in any cell type through the excision of a floxed *neo* cassette by Cre, i.e., $R26R^{neo}$ gives $R26R^{lacZ}$ (Soriano, 1999). LacZ activity was determined by staining with X-gal (Hogan *et al.*, 1993).

Mouse Strains

The inbred strain MB6 produced in our laboratory is now at F22. It is homozygous for the tyrosinase, Tyr^c (albino), non-agouti, *a* (black), and tyrosine-related protein 1, $Tyrp1^b$ (brown) coat color alleles, and the glucose phosphate isomerase 1, $Gpi1^b$, allele. It was produced by crossing the outbred strain MF1 (Harlan, Indianapolis, IN) with C57BL/6J then backcrossing to MF1 three times before initiating brother-sister mating. (MB6 x C57BL/6J-Tyr^{c-2J})F_1 females produce large numbers of eggs on superovulation.

The $Hprt^{cre}$ mutation is also being bred into a congenic 129S1 strain produced in our laboratory—129S1.B6-*a* (N10) or black 129S1 mice. This will allow for assessment of germ-line transmission by coat color when male chimeras are mated to 129S1.B6-*a* (N10) $Hprt^{cre}$/+ females.

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